

drafted to avoid the informalities of the canceled claims.

New claim 162 corresponds to claim 122 combined with canceled claim 123. Claims 163-169 correspond respectively to canceled claims 124-130, while claim 170 corresponds to canceled claim 131 except for the deletion of the term "optionally" in step (iii).

Claim 171 corresponds to canceled claim 132, and claim 172 corresponds to canceled claim 133 except that the term "optionally" in step (iii) has been deleted. New claims 173-182 correspond to canceled claims 134 -143. Claim 183 corresponds to the combined subject matter of canceled claims 144 and 145.

Claims 184-190 correspond to canceled claims 146-152 and claim 191 corresponds to canceled claim 153 except for the deletion of the term "optionally" in step (iii). Claim 192 corresponds to canceled claim 154. Claim 193 corresponds to canceled claim 155 except that the term "optionally" in step (iii) has been deleted. Claim 194 corresponds to canceled claim 156 and claim 195 corresponds to the combined subject matter of canceled claims 157 and 123.

Claim 196 corresponds to the combined subject matter of canceled claims 158 and 123. Claim 197 corresponds to the subject matter of claims 159 and 123. Claim 198 corresponds to the combined subject matter of canceled claims 160 and 123. Claim 199 corresponds to the combined subject matter of canceled claims 161 and 123.

The newly presented claims point out the biological material wherein the endothelial, glandular, skin adnexa and germinative cells are cocultured with fibroblasts or they are cultivated in the presence of fibroblasts.

The data set forth in the examples of the present specification clearly demonstrate that it is essential for successfully culturing weak and fragile cells like those contemplated in the present invention, that the culturing procedure be carried out in the presence of a support based on a hyaluronic acid derivative like the one listed in the new main claim 162, but also a co-cultivation with fibroblast or at least the presence of a medium treated with fibroblasts.

Example 2 reports that when endothelial cells from the umbilical vein, that are extracted as disclosed in Example 1, are first amplified on gelatin treated dishes and then seeded on membranes of nonwoven fabric (HYAFF<sup>®</sup>) in 24 well dishes at a density of 30,000/cm<sup>2</sup> in different culture conditions:

with a medium enriched with growth factors,

with a medium treated with human fibroblasts three days before seeding,

the endothelial cells show an increase in the rate of proliferation as reported in Fig.1. The graph of Fig. 1 shows that the rate of proliferation increases to a high degree in a culture medium supplemented with fibroblasts on the biomaterial made of HYAFF 11 over a period of 96 hours (the absorbance value is over 0.30) and in any case is already considerably high at 24 hours (about 0.20). The rate of proliferation decreases over a period of 96 hours in cells seeded on the same material, but in the presence only of growth factors, in fact in Fig. 1, the absorbance decreases from a value of 0.10 at 24 hours from about 0.05 at 96 hours.

By contrast in comparative Example 3, HUVEC was cultured in a manner like the procedures of Example 2 with the only

difference being that these cells were cultured on collagen coated plates, instead of a support of HYAFF.

The results reported in Fig. 2 clearly indicate that although there is an increase in the rate of proliferation over a period of 96 hours from 0.05 at 24 hours to about 0.10 at 96 hours, **the growth values are notably lower** than those obtained with the sample of non woven Hyaff 11 supplemented with fibroblasts treated medium.

The successive examples confirm the above results and in particular demonstrate that even better results are obtained:

when HUVEC cells are co-cultured with fibroblasts (Examples 4 and 5) and in presence of human fibroblasts only for 4 weeks (Example 5),

when liver cells are cocultured on non woven HYAFF with human dermal fibroblasts seeded on the biomaterial 7 weeks before, (Example 6),

when islet of Langerhan's cells are cocultured with human dermal fibroblasts seeded on the biomaterial 7 days before the islet of Langerhans (Example 7),

when hair bulbs are cocultured with human skin fibroblasts seeded on biomaterial respectively 3 and 5 weeks before the hair bulb cells (Example 8).

Claims 122-130, 132, 134-152, 154, 156 and 159-160 were rejected under 35 U.S.C. §112, second paragraph, for failing to particularly point out and distinctly claim the subject matter that the applicant regards as the invention.

Reconsideration is requested.

The newly presented claims replace the expression "an O- or an N-sulphated hyaluronic acid" with --an O-, an N-sulphated hyaluronic acid and a derivative thereof--. In view of this Amendment, it is requested that this ground of rejection be withdrawn.

Claims 122, 126, 128-129, 134, 138-141, 144, 148, 150-151, and 156-161 were rejected under 35 U.S.C. §102(b) as being anticipated by Bellini et al.

The newly presented main claims point out a biological material containing the above described weak endothelial, liver and Langerhans' cells and a support which comprises a hyaluronic acid derivative. The cells are cocultured with fibroblasts or they are cultivated in the presence of fibroblasts. This clearly defined biological material is decidedly **not anticipated by Bellini not only because it is grown on a different type of support, but also because it encompasses a further cellular component not disclosed by Bellini.** For these reasons, the rejection for anticipation based on Bellini should not be applied to reject the newly presented claims.

Claims 122, 128-130, 134, 139-144, 150-152 and 156-161 were rejected under 35 U.S.C. §102(b) as being anticipated by Abatangelo et al. as evidenced by della Valle et al. or Dorigatti et al.

Reconsideration is requested.

The biological material, as defined in the new set of claims, and in particular in the new main claims is novel and unobvious from the biologic material disclosed in the Abatangelo patent. The autologous or homologous bone marrow stem cells of Abatangelo which are partially or completely differentiated into connective tissue specific cells are distinctly different from the cells in the claimed biological material.

Abatangelo does **not** suggest that the stem cells which are partially or completely differentiated into endothelial cells can be treated with a medium containing fibroblasts or can be cocultured with fibroblasts.

Since the newly presented claims point out that the claimed biological material is prepared from a medium that is treated with fibroblasts or is cocultured with fibroblasts, it is readily apparent that the newly presented claims point out novel and unobvious subject matter that is not found in the prior art and it is requested that this ground of rejection be withdrawn.

Claims 122, 129-130, 144 and 151-152 were rejected under 35 U.S.C. §103(a) as being unpatentable over Bellini et al, in view of Cialdi et al.

Reconsideration is requested.

It has been noted supra that Bellini et al combined with the teachings of Cialdi does not anticipate the newly presented claims to the biological materials new set of claims.

Bellini simply and generically states that the

specific hyaluronic acid derivative may be used as support for a long list of cells namely fibroblasts, keratinocytes, osteocytes, stem cells, endothelial cells, Kupfer's and Langerhans cells. Nowhere in Bellini, can the skilled person detect a scintilla of a teaching that it was possible to obtain an efficient growth of the presently claimed cells which are very **weak and fragile** and are characterized by having **very short survival times**, either by the addition of a medium treated with fibroblasts or in alternative by coculturing said cells with fibroblasts. The above deficiency is not overcome by Cialdi because Cialdi merely discloses the sulphated derivative of class (C), namely one of the materials used as the support in the presently claimed material.

Although Cialdi mentions material C in a three-dimensional structure, Cialdi does not envisage the use of said material **as a support** for the cells utilized in the present invention. Cialdi does not provide the slightest hint that the presence of fibroblasts in a coculture or contained in a medium could provide a favorable impact on the proliferation of the aforesaid cells. For these reasons, it is requested that the rejection based on Bellini in view of Cialdi not be applied to the newly presented claims.

Claims 122, 125, 131, 133, 144, 147, 153 and 155 were rejected under 35 U.S.C. §103(a) as being unpatentable over Bellini in view of Williams et al.

Reconsideration is requested.

The newly presented claims have rendered this ground of rejection moot. The combined teachings of Bellini and Williams et al. do not suggest a biological material comprising the cells lines of new claim 162, cultivated in the presence of fibroblasts, in combination with a hyaluronic acid derivative.

Such a biological material could not have been obtained by following the teachings of the cited references. For these reasons, it is requested that this ground of rejection not be applied to reject the newly presented claims.

Any additional fee that is required in connection with this amendment may be charged to Deposit Account No. 08-1540.

An early and favorable action is earnestly solicited.

Respectfully submitted,



James V. Costigan

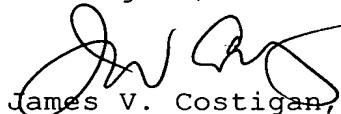
Registration No: 25,669

MAILING ADDRESS,

Hedman & Costigan, P.C.  
1185 Avenue of the Americas  
New York, NY 10036  
(212) 302-8989

It is hereby certified that this Amendment is being deposited with the United States Postal Service as first class mail postage prepaid in an envelope addressed to:

Commissioner for Patents  
Washington, DC 20231



James V. Costigan, Reg. No. 25,669